UNIVERSITI PUTRA MALAYSIA

A SYSTEMATIC REVIEW ON UTERINE MICROBIOTA OF DAIRY COWS ASSOCIATED WITH METRITIS, CLINICAL AND SUBCLINICAL ENDOMETRITIS

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan

DECEMBER 2021

DEDICATIONS

This review paper is dedicated to my family, my supervisor, my friends and all large animal practitioners that would find it useful.

ACKNOWLEDGEMENTS

All praise is due to Allah for giving me the strength I need to complete this Final Year Project thesis.

First and foremost, my heartfelt gratitude goes to my one and only supervisor, Assoc. Prof. Dr. Nurhusien Yimer for all the knowledge and guidance throughout this study. Without him, it would be impossible for me to complete my final year project.

A special thanks to my dearest friends Arinah Nabihah Ahmad, Tharishinie Murugapiran, Syakirah Abdul Razak, Nurul Syahira Kaswadinata, Shariffah Salmiah Syed Mohd and Nurazila Hani Mat Johari for always being there for me whenever I need a helping hand. Not to forget Iskandar Zulkarnain Idris for supporting me mentally and emotionally.

I would like to thank my father, Khairul Faizy Abdullah and my mother, Zuriati Zainuddin for raising me into the person I am today. And lastly, to my siblings, Nurul Batrisyia, Nurul Yasmeen, Muhammad Darwish and my cats Abul, Cherry, Simba and Olaf for making me happy.

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LIST OF ABBREVIATIONS

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analyses
AST	Antimicrobial Susceptibility Testing
°C	Degree Celcius
%	Percentage
RQDT	Research Question Development Tool
M	Metritis
CE	Clinical Endometritis
SCE	Subclinical Endometritis
BCS	Body Condition Score
APM	Acute Puerperal Metritis

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD4999 - Projek Tahun Akhir

KAJIAN SISTEMATIK MENGENAI MIKROBIOTA RAHIM LEMBU TENUSU YANG BERKAITAN DENGAN METRITIS, ENDOMETRITIS KLINIKAL DAN SUBKLINIKAL

Oleh

NURUL MAISARAH BINTI KHAIRUL FAIZY

2021

Penyelia: Prof. Madya Dr. Nurhusien Yimer Degu

Lembu selepas bersalin biasanya mengalami penyakit rahim seperti metritis dan endometritis. Metritis dan endometritis boleh menyebabkan kerugian ekonomi dalam kalangan penternak kerana ia memberi kesan negatif kepada prestasi pembiakan lembu tenusu. Walaupun beberapa kajian telah dilaporkan, terdapat kekurangan pemahaman yang jelas mengenai bakteria rahim yang paling kerap dilaporkan yang dikaitkan dengan metritis, endometritis klinikal dan subklinikal. Objektif kajian ini adalah untuk mengkaji secara sistematik dan mengenal pasti mikrobiota rahim lembu tenusu yang dikaitkan dengan metritis, endometritis klinikal dan subklinikal dari kawasan geografi yang berbeza berserta spektrum sensitiviti antimikrob dan faktor risiko yang paling penting. Berpandukan Item Pelaporan Pilihan untuk Kajian

Sistematik dan Meta-analisis (PRISMA) 2020, semakan sistematik telah dijalankan ke atas mikrobiota rahim lembu tenusu dengan metritis, endometritis klinikal dan subklinikal menggunakan kajian yang diterbitkan dari 2012 hingga 2021 dalam pangkalan data *SCOPUS* dan *Google Scholar*. Terdapat sejumlah 36 kertas kerja yang diterbitkan disertakan untuk penilaian penuh. Daripada analisis tematik, ulasan ini mengemukakan lima tema utama yang merangkumi (1) kaedah persampelan; (2) pengagihan mikrobiota; (3) taburan geografi; (4) faktor risiko dan (5) dapatan Ujian Kecenderungan Antimikrob (AST). *Trueperella pyogenes* dan *Escherichia coli* adalah antara isolat yang biasa ditemui dalam semua kajian yang disemak. *Trueperella pyogenes* didapati sangat berkait dengan endometritis klinikal manakala *Escherichia coli* dikaitkan dengan kebanyakan kes endometritis subklinikal. Sementara itu, tinjauan menunjukkan bahawa pelbagai jenis bakteria boleh menyebabkan metritis pada lembu. Kajian sistematik ini memberikan gambaran keseluruhan pengetahuan semasa dan perbandingan yang jelas tentang mikrob rahim lembu tenusu yang mempunyai metritis, endometritis klinikal dan subklinikal dari kawasan geografi yang berbeza. Penemuan ini akan membantu kajian lanjut pada masa hadapan mengenai langkah terapeutik, pencegahan dan kawalan untuk metritis, endometritis klinikal dan subklinikal dalam lembu tenusu, dengan itu mengurangkan kerugian kewangan yang berkaitan oleh penternak.

Kata kunci: *mikrobiota rahim, lembu tenusu, metritis, endometritis klinikal, endometritis subklinikal*

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in
partial fulfilment of the course VPD 4999 – Final Year Project

A SYSTEMATIC REVIEW ON UTERINE MICROBIOTA OF DAIRY COWS ASSOCIATED WITH METRITIS, CLINICAL AND SUBCLINICAL ENDOMETRITIS

by

NURUL MAISARAH BINTI KHAIRUL FAIZY

2021

Supervisor: Assoc. Prof. Dr. Nurhusien Yimer Degu

Postpartum cows commonly develop uterine diseases such as metritis and endometritis. Metritis and endometritis can lead to economic losses among farmers as it negatively affects the reproductive performances of dairy cows. Despite several studies reported, there is a lack of clear understanding on the most frequently reported uterine bacteria associated with metritis, clinical and subclinical endometritis. The objective of this study is to systematically review and identify the uterine microbiota of dairy cows associated with metritis, clinical and subclinical endometritis from different geographical areas along with their antimicrobial sensitivity spectrum and the most important risk factors. Guided by Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) 2020, a systematic review has been conducted on uterine microbiota of dairy cows with metritis, clinical and subclinical endometritis using studies published from 2012 until 2021 in SCOPUS and Google Scholar databases. There were a total of 36 published papers included for a full assessment. From the thematic analysis, this review presented five main themes which include (1) sampling methods; (2) microbiota distribution; (3) geographical distribution; (4) risk factors and (5) Antimicrobial Susceptibility Testing (AST) findings. *Trueperella pyogenes* and *Escherichia coli* are among the isolates that were commonly found across all the studies reviewed. *Trueperella pyogenes* was found to be highly associated with clinical endometritis while *Escherichia coli* was linked to most of the subclinical endometritis cases. Meanwhile the review showed that various types of bacteria can cause metritis in cows. This systematic review provides an overview of a current knowledge and a clear comparison on the uterine microbes of dairy cattle having metritis, clinical and subclinical endometritis from different geographical areas. The findings would aid further studies in future on therapeutic, preventive and control measures for metritis, clinical and subclinical endometritis in dairy cows, thereby reducing related financial losses by farmers.

Keywords: *uterine microbiota, dairy cows, metritis, clinical endometritis, subclinical endometritis*

1.0 INTRODUCTION

Bacterial contamination of the endometrium after parturition can lead to uterine infection, resulting in postpartum uterine diseases. This could happen in most of the postpartum cattle when bacteria from the animal surface and the environment infect the lumen of the uterus (Sheldon et al., 2006). This condition is commonly observed in dairy cows such as in Holstein-Friesian cows. Clinical uterine diseases can cause subfertility and infertility, which markedly affects cattle reproductive health (Sheldon & Owens, 2017). This could lead to reproductive failure, thereby reducing reproductive performances of the cattle and resulting in economic losses among dairy cattle farmers.

Metritis and endometritis are the two major postpartum clinical conditions (Sheldon & Owens, 2017). There are two types of metritis, namely puerperal metritis and clinical metritis. Puerperal metritis is known as an acute systemic bacterial infection of the uterus which usually occurs within 10 days postpartum. It is also proposed that a puerperal metritis case should be determined when an animal with a fetid watery reddish brown uterine discharge and an unusual uterus enlargement, pertaining to systemic signs such as decrease in milk production, dullness or other signs of toxemia, and fever of >39.5 °C occurring within 21 days after parturition (Sheldon et al., 2006). Healthy animals within 21 days post parturition may be graded as having clinical metritis when they are having an unusual uterus enlargement and a detectable purulent uterine discharge in the vagina (Sheldon et al., 2006).

Sheldon et al., (2006) concluded that clinical endometritis in a cow can be defined by the presence of purulent uterine discharge which can be detected in the vagina at 21 days or more after parturition, or by the presence of detectable mucopurulent discharge in the vagina after 26 days post parturition. The same report proposed that a cow having subclinical endometritis can be determined by uterine cytology samples collection 21–33 days after parturition, which would show presence of >18% neutrophils, or presence of >10% neutrophils at 34–47 days, without having any symptoms of clinical endometritis.

Although elimination of bacterial contamination depends on the process of uterine involution, endometrium regeneration, and defense mechanisms of the uterus (Sheldon et al., 2006), it is very important to identify the microbes that would cause metritis, clinical and subclinical endometritis in order for the farmers to plan the appropriate preventive and control measures as this would help to reduce the occurrence of those clinical conditions.

However, there is a lack of clear understanding on the most frequently reported uterine bacteria associated with metritis, clinical and subclinical endometritis, despite reports from several studies. According to Takamtha et al. (2013), bacterial findings and antimicrobial susceptibility reports associated with endometritis in dairy cattle in Thailand were very little and not updated. Similarly for other reports, the studies made were focused on certain farm areas, thus the bacterial findings were found in a sporadic manner. Other than the lack of information on the predominant risk factors and antimicrobial sensitivity spectrum, there was also no systematic review that has been done on this topic.

Therefore, this study was conducted to systematically review and identify the uterine microbiota of dairy cows associated with metritis, clinical and subclinical endometritis from different geographical areas along with their antimicrobial sensitivity spectrum and the risk factors pertaining to the uterine clinical conditions.

2.0 MATERIALS AND METHODS

2.1 Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA)

This review follows the guide from a publication standard called PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analyses) statement. PRISMA statement was published in 2009 with the aim to enable authors to prepare a complete, accurate and transparent review that is beneficial to the users and tackle poor reporting of systematic reviews (Page et al., 2021). PRISMA 2009 statement is replaced with PRISMA 2020 statement which includes new reporting guidelines and has been made particularly for systematic reviews of studies that appraise health interventions effects (Page et al., 2021). PRISMA statement is suitable to be used in this systematic review as it offers the following three advantages: (1) research question can be defined clearly; (2) the inclusion and exclusion criteria are able to be identified, and (3) highest number of relevant and accessible scientific literature can be assessed and examined within a specific period of time (Shamseer et al., 2015).

2.2 Formulation of research question

Formulation of a concise and clear research question serves as an important phase to guide the authors in many aspects of the systematic review writing which includes identification of suitable keywords, determining eligible criteria, systematic literature search, data collection and extraction from the selected studies and result presentation. The research question for this study was formulated based on PICo. PICo is a Research Question Development Tool (RQDT) which guides authors to create research questions that are suitable for their reviews. The three main concepts of PICo are

Population or Problem, Interest and Context. In the present review, effort has been made to incorporate these three aspects based on the concepts mentioned earlier which include: metritis, clinical and subclinical endometritis (Problem), uterine microbiota (Interest) and dairy cows (Context) used to develop the main research question – What are the uterine microbiota in dairy cows associated with metritis, clinical and subclinical endometritis?

2.3 Systematic Literature Search

2.3.1 Identification

The first step in systematic literature search is identification process which involves determination of suitable keywords to search for sufficient information. Based on the research question developed, a few main keywords were recognized which are microbiota, dairy cows, metritis, clinical endometritis and subclinical endometritis. The authors searched for the synonyms of the main keywords and the related terms in order to broaden the search result by using online thesaurus, referring to the keywords that were suggested by Scopus and seeking the opinion from an expert. These keywords were combined and processed using search functions such as Boolean operators and truncation to form search strings. Therefore, the following search strings were used in the selected databases which are Scopus and Google Scholar on 26 September 2021 (Table 1). From this process, there were a total of 258 articles from Scopus and 16 articles from Google Scholar databases identified.

Table 1: Search string used in the selected database.

Database	String
Scopus	TITLE-ABS-KEY ((“microbiota” OR “microorganism*” OR “microbe*” OR “bacteria”) AND (“cow*” OR “cattle” OR “bovine” OR “heifer” OR “dairy cow*” OR “dairy cattle”) AND (“clinical endometritis” OR “subclinical endometritis” OR “metritis” OR “cytological endometritis” OR “endometrial cytology”))
Google Scholar	allintitle: (“microbiota” OR “microorganisms” OR “bacteria”) AND (“cows” OR “cattle”) AND (“clinical endometritis” OR “subclinical endometritis” OR “metritis” OR “cytological endometritis” OR “endometrial cytology”)

2.3.2 Screening (Inclusion and Exclusion Criteria)

The next step is screening where inclusion and exclusion criteria will be decided by the author to select the articles that are appropriate to be included in the systematic review. The screening process was done by using the filter feature which is available in Scopus and Google Scholar. This process had removed six duplicated articles, which left 268 documents to be screened. There were a number of inclusion and exclusion criteria involved in this process. The first inclusion criterion was the document type where the author would only accept published articles. Other document types such as review articles, chapters in books, books, and conference proceedings were all excluded. Next inclusion criterion was the duration of study. The authors had selected the articles that were only published within the period of 2012 to 2021. This timeline was chosen as the number of studies published within these 10 years would be sufficient to produce a review that is representable. The last criterion for inclusion was language. Only English language articles were included in this study to avoid any difficulties or confusion to do data extraction. From these screening process, a total of 124 documents were found unfit and removed, leaving 144 documents for further screening.

2.3.3 Eligibility

After identification and screening, the remaining articles were assessed for eligibility according to some specific criteria. Articles that did not meet the author's criteria were excluded from the study after reading through the abstract, methodology and results from the articles. The author selected the studies that include details on bacteria types present in cases of cows with metritis, clinical or subclinical endometritis. The studies should also be done on dairy cows with samples taken from the uterus. 29 studies which were not done in dairy cow with endometritis and 15 studies using samples other than uterine sample were excluded. Meanwhile, 55 studies provided insufficient information and three articles were inaccessible. Other than that, three studies done in treated cows and three studies with induced endometritis were also removed from this process. A total of 108 articles were excluded out of 144 articles remaining for eligibility process which resulted in 36 articles to be included for this study.

2.4 Data Extraction and Analysis

After the eligibility process, the 36 articles included in this study were assessed, reviewed, and examined. A spreadsheet form was generated before reviewing the included articles to collect the general data of the articles including the authors, title, journal and publication year. Data extraction of each article was done by scanning through the abstracts, methods and results followed by the full-text to search and collect all the data that are accessible. The data that were extracted are as follows: first author, year of publication, cattle breed, geographical distribution of the papers, sampling methods, sample types, types of condition, microbiota detected, Antimicrobial Susceptibility Testing (AST) findings and risk factors to microbial detection.

A thematic analysis of the available data for this review was conducted to analyse all the collected data into a number of themes and sub-themes. The data extracted were converted into beneficial data and directed by distinguishing the concepts and themes that link and connect all the obtainable data from the papers included. The themes decided were: (1) sampling methods - categorised the methods for bacteriology for every article; (2) microbiota distribution - categorised the bacterial isolates found in dairy cows with metritis, clinical and subclinical endometritis; (3) geographical distribution - summarised the countries for each studies and the countries for the major bacterial isolates; (4) risk factors - summarised factors associated with metritis, clinical and subclinical endometritis investigated in some of the papers, and finally (5) AST findings - summarised bacterial isolates with their AST profiles from some of the studies. Any further analysis and re-assessment of the themes and sub-themes were performed continuously.

3.0 RESULTS

3.1 Summary of Systematic Literature Search

A total of 274 articles were obtained from two databases, where 258 studies were identified from Scopus and 16 studies were found in Google Scholar using the keywords search string. As shown in Figure 1 below, a stepwise screening and exclusion process were undertaken to determine the eligible studies to be used for the systematic review. Six articles were identified as duplicates and removed, leaving 268 articles to be screened. By using the automation tools in the databases to filter out review articles, chapters in books, books, and conference proceedings, articles published earlier than 2012, and non-English articles, 124 articles were excluded automatically. Following title, abstract, methods and results screening of the 144 articles left, a total of 108 studies were excluded manually from the eligibility process for failing to meet the inclusion criteria. Subsequently, only 36 studies were included for this review.

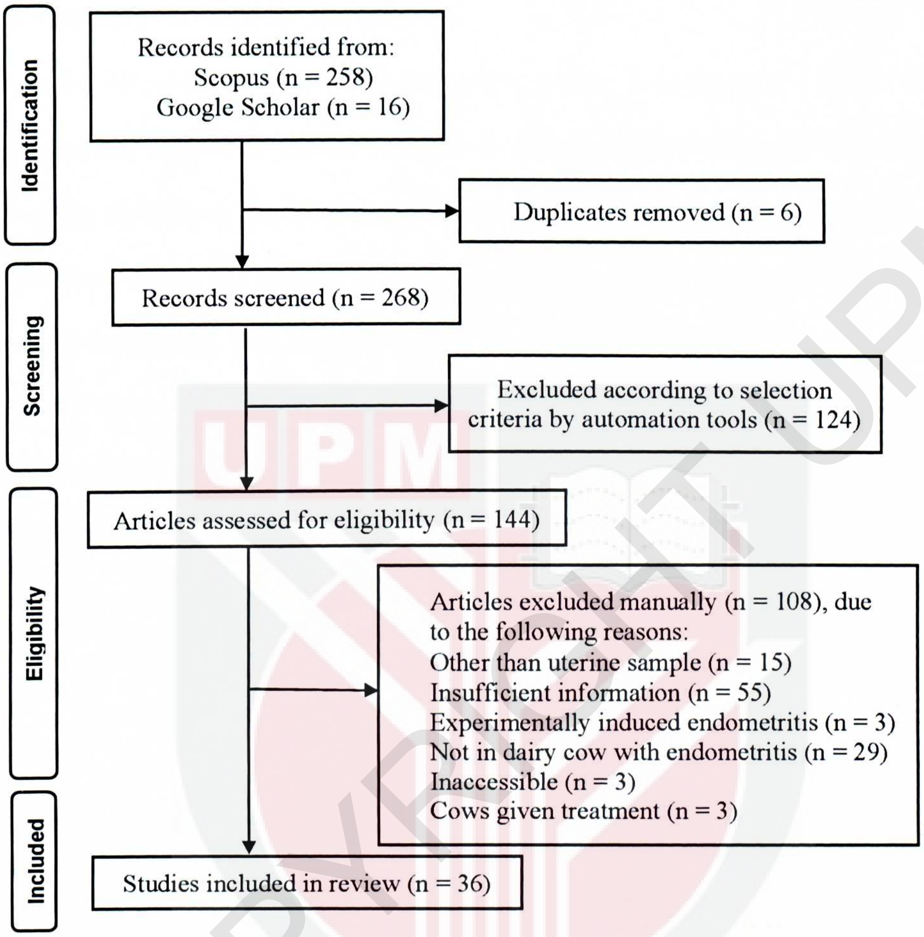

Figure 1: Flow diagram following the PRISMA guidelines (adapted from Page et al., 2021), showing the process of systematic literature search.

3.2 Sampling Methods for Bacteriology

From the 36 published papers included in this review, nine papers use cytobrush as the sampling method as shown in Figure 2. 16 papers apply swab for bacteriology sampling while flush fluid and uterine secretion were used in four published studies each. Three papers did multiple methods for sampling which includes the use of both swab and cytobrush and combination of uterine secretion, flush fluid and biopsy. Figure 3 shows the different sampling methods that were employed across different clinical conditions, namely metritis, clinical and subclinical endometritis, and mixed condition.

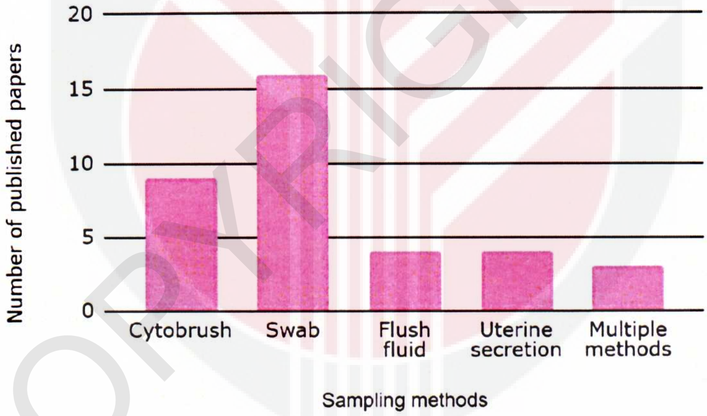

Figure 2: Distribution of sampling methods for bacteriology used in the published papers. Note: multiple = combination of more than one method

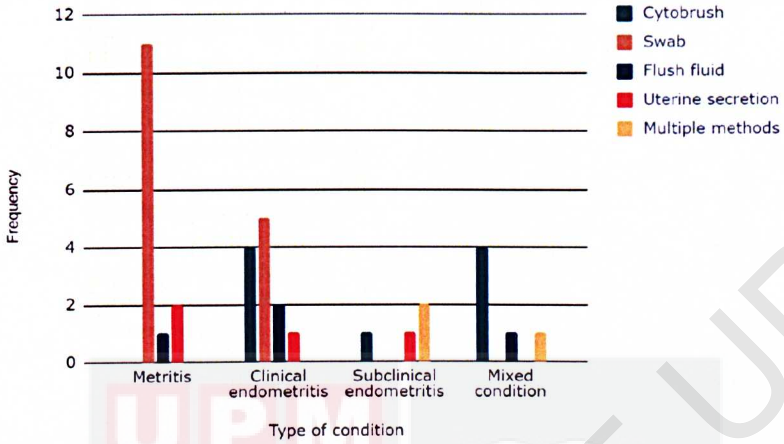

Figure 3: Frequency distribution of sampling methods employed across different clinical conditions.

3.3 Microbiota Distribution Associated with Metritis, Clinical Endometritis and Subclinical Endometritis

There are approximately 72 different species of microbes discovered by all of the published papers included in this review. Due to the different reporting methods by the papers, two types of tables are formulated accordingly as shown in Table 2 and Table 3. As seen in Table 2, a study by Kasimanickam et al. (2016) shows that *T. pyogenes* and *E. coli* were found in 100% of the cows with metritis. While in Table 3, one of the top findings was reported by Ordell et al. (2016) which shows that 86% of *E. coli* was discovered in metritic cows.

Table 2: Occurrence of microbes among cows studied in different clinical conditions.

Clinical condition	**Microbial type**	**Occurrence**	**Studies**
Metritis	*Trueperella pyogenes* *Escherichia coli* *Vibrio* spp.	66.67% (4/6) 16.67% (1/6) 16.67% (1/6)	(Mileva et al., 2020)
Metritis	*T. pyogenes*	23.83% (41/172)	(Rezanejad et al., 2019)

Metritis	*E. coli* *Staphylococcus* spp. *Klebsiella* spp.	28.57% (4/14) 21.43% (3/14) 21.43% (3/14)	(Yadav & Jadhav, 2018)
Metritis	*Trueperella Pyogenes* *E. coli* *Fusobacterium necrofurom* *Staphylococcus intermedius* *Streptococcus a hemolytic* *Proteus mirabilis* *Entrococcus faecalis* *Pseudomonas aerogenosa* *Actinobacter* spp. *Klebcilla* *Corynebacterium* spp. *Staphylococcus aureus* *Serratia marcescens* *Pasteurella multocida*	8% (16/200) 7% (14/200) 5% (10/200) 2.5% (5/200) 2% (4/200) 1.5% (3/200) 1.5% (3/200) 1% (2/200) 1% (2/200) 1% (2/200) 0.5% (1/200) 0.5% (1/200) 0.5% (1/200) 0.5% (1/200)	(Ashrafi Tamai et al., 2018)
Metritis	*T. pyogenes* *E. coli*	100% (5/5) 100% (5/5)	(Kasimanickam et al., 2016)
Metritis	*Trueperella pyogenes* *Escherichia coli* *Clostridium* spp. Gram positive aerobes (*Streptococcus* spp., *Staphylococcus* spp., *Micrococcus* spp.) Gram positive anaerobes (*Peptostreptococcus* spp.) Gram negative aerobes (*Pasteurella* spp., *Proteus* spp., *Actinobacillus* spp.) Gram negative anaerobes (*Fusobacterium* spp., *Bacteroides* spp., *Prevotella* spp., *Porphyromonas* spp.)	88.24% (15/17) 52.94% (9/17) 23.53% (4/17) 70.59% (12/17) 64.71% (11/17) 17.65% (3/17) 58.82% (10/17)	(Credille et al., 2014)
CE	*Trueperella pyogenes*	26.5% (22/83)	(Paiano et al., 2021)
CE	*Bacillus* spp., *Corynebacterium* spp.	(22.6%; 14/62) (9.4%;12/62)	(Ballas et al., 2021)
CE	*Trueperella pyogenes* *Streptococcus* spp. *Acinetobacter Iwoffii* *Bacteroides pyogenes* *Bacillus* spp. *Peptoniphilus indolicus* *Proteus mirabilis*	80% (4/5) 20% (1/5) 20% (1/5) 20% (1/5) 20% (1/5) 20% (1/5) 20% (1/5)	(Pascottini et al., 2020)
CE	*T. pyogenes* *E. coli*	62.5% (10/16) 56.25% (9/16)	(Kasimanickam et al., 2016)
CE	*T. pyogenes* *Strep. pyogenes* non-haemolytic *Streptococcus* spp	22.22% (2/9) 11.11% (1/9) 11.11% (1/9)	(Ledgard et al., 2015)

	Strep. agalactiae Mixed *Staph.* species with *E. coli* haemolytic *Staph.* species	22.22% (2/9) 11.11% (1/9) 22.22% (2/9)	
CE	*T. pyogenes* *E. coli* *F. necrophorum*	64.71% (11/17) 23.52% (4/17) 11.76% (2/17)	(Aghamiri et al., 2014)
CE	*Trueperella pyogenes* CNS	25% (4/16) 12.5% (2/16)	(Madoz et al., 2014)
CE	*T. Pyogenes* *E. Coli* *Streptococcus* spp *Pasteurella* spp *T. Pyogenes* in association with *E. Coli* and *Streptococcus* spp.	46.15% (12/26) 3.85% (1/26) 38.46% (10/26) 3.85% (1/26) 7.69% (2/26)	(Mari et al., 2012)
CE	*Trueperella pyogenes* *Escherichia coli* *Pasteurella* spp. *Pseudomonas* spp. *Corynebacterium* spp. *Acinetobacter* spp.	60.78% (31/51) 29.41% (15/51) 3.92% (2/51) 1.96% (1/51) 1.96% (1/51) 1.96% (1/51)	(Brick et al., 2012)
SCE	*Staphylococcus* spp. *Aerococcus viridans* *Corynebacterium* spp. *Streptococcus* spp.	50% (4/8) 12.5% (1/8) 25% (2/8) 12.5% (1/8)	(Pascottini et al., 2020)
SCE	*Citrobacter* sp. *Escherichia coli* Coagulase negative *staphylococci* *Streptococcus* sp. *Candida* sp. *Klebsiella* sp. *Aspergillus* sp.	5% (2/40) 20% (8/40) 2.5% (1/40) 2.5% (1/40) 2.5% (1/40) 2.5% (1/40) 2.5% (1/40)	(Ağaoğlu et al., 2020)
SCE	*Ureapasma diversum*	16% (3/19)	(Díaz et al., 2019)
SCE	*Escherichia coli* *Histophilus somni*	26.67% (4/15) 13.33% (2/15)	(Peter et al., 2018)
SCE	*T. pyogenes* *E. coli*	25% (2/8) 50% (4/8)	(Kasimanickam et al., 2016)

Table 3: Occurrence of microbes by percentage of isolates studied in different clinical conditions.

Clinical condition	**Microbial type**	**Occurrence**	**Studies**
Metritis	*Bacteroides* spp. *Porphyromonas* spp. *Fusobacterium* spp.	41.18% 9.23% 20.81%	(Chen et al., 2020)

Metritis	*Fusobacterium necrophorum*	39.10%	(Jeon, Ma, et al., 2016)
	Porphyromonas levii	9.27%	
	Bacteroides heparinolyticus	3.33%	
	Bacteroides denticanum	4.94%	
	Porphyromonas somerae	7.25%	
	Helcococcus ovis	4.16%	
	Bacteroides pyogenes	0.27%	
	Peptoniphilus asaccharolyticus	3.20%	
	Fusobacterium spp.	0.18%	
	Parvimonas micra	4.55%	
	Prevotella spp.	2.50%	
	Peptostreptococcus anaerobius	1.31%	
	Clostridiisalibacter bacterium	0.43%	
Metritis	*Trueperella pyogenes*	21%	(Ordell et al., 2016)
	Escherichia coli	86%	
	Gram positive cocci (*streptococci* and *enterococci*)	33%	
	Fusobacterium necrophorum	24%	
	Clostridium spp.	18%	
	Other Gram-negative (*Enterobacter* spp., *Acinetobacter* spp., *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri* and *Aeromonas hydrophila*)	12%	
	Pasturella spp.	5%	
CE	*Trueperella pyogenes*	2.36%	(Wang et al., 2018)
	Lactococcus piscium	31.41%	
	Bacillus sp.	10.45%	
	Lactococcus lactis subsp. lactis	5.45%	
	Solibacillus silvestris StLB046	4.21%	
	Pseudomonas sp.	3.14%	
	Arthrobacter sp	3.65%	
	Fusobacterium necrophorum	10.58%	
	Uncultured *Porphyromonas* sp.	6.52%	
	Lysinibacillus sp.	1.19%	
	Bacillus oceanisediminis	0.91%	
	Carnobacterium maltaromaticum	0.71%	
	Exiguobacterium sp. AT1b	0.69%	
	Streptococcus sp.	0.68%	
	Parvimonas.uncultured bacterium	2.24%	
	Streptococcus salivarius subsp. thermophilus	0.49%	
		0.41%	
		0.35%	

	Psychrobacter sp.	1.49%	
	Pseudomonas vranovensis	0.29%	
	Helcococcus ovis	0.24%	
	Brochothrix thermosphacta	0.26%	
	Oceanobacillus profundus	0.17%	
	Escherichia-Shigella sp.	0.15%	
	Moraxella osloensis		
	Ruminococcaceae.uncultured	0.18%	
	sp.	0.13%	
	Leuconostoc sp.	0.18%	
	Lactobacillus helveticus	0.66%	
	Leuconostoc lactis	0.40%	
	Peptoniphilus indolicus	0.11%	
	Ureaplasma diversum		
	Acinetobacter sp.		
CE	*Staphylococcus* spp.	19.7%	(Schlegl et al., 2020)
	Bacillus spp.	12.6%	
	Streptococcus spp.	10.2%	
	Corynebacterium spp.	8.7%	
	Lysinibacillus spp.	7.9%	
	Micrococcus spp.	5.5%	
	Acinetobacter spp.	4.7%	
	Brevibacterium spp.	4.7%	
	Escherichia coli	3.9%	
	Pseudomonas spp.	3.1%	
CE	*Trueperella pyogenes*	25%	(Gurunathan et al., 2018)
	Prevotella melaninogenica	30%	
	Escherichia coli	20%	
	Streptococcus spp.	15%	
	Staphylococcus spp.	10%	
	Campylobacter fetus	8%	
	Klebsiella spp.	5%	
	P. aeruginosa	3%	
	Clostridium spp.	1%	
CE	*Staphylococcus* spp.	44.8%	(Zhao et al., 2014)
CE	*Trueperella pyogenes*	15%	(Liu et al., 2013)
	Staphylococcus aureus	21.8%	
	Streptococcus sp.	19.2%	
	Escherichia coli	19.2%	
	Fusobacterium necrophorum	7.7%	
	Prevotella melaninogenica	6.4%	
	Proteus sp.	5.6%	
	Bacillus sp.	6.8%	
	Bacteroides sp.	4.3%	
CE	*Trueperella pyogenes*	14%	(Takamtha et al., 2013)
	Corynebacterium spp.	18%	
	Staphylococcus spp.	11%	
	Streptococcus spp.	9%	
	Bacillus spp.	5%	
	Micrococcus spp.	1%	
	Escherichia coli	24%	

	Pasteurella spp.	6%	
	Actinobacillus spp.	3%	
	Enterobacter spp.	3%	
	Achromobacter spp.	2%	
	Klebsiella spp.	1%	
	Proteus spp.	1%	
	Citrobacter spp.	1%	
	Erysipelothrix spp.	1%	
SCE	*Lactococcus piscium*	41.41%	(Wang et al., 2018)
	Bacillus sp.	14.92%	
	Lactococcus lactis subsp. lactis	7.10%	
	Solibacillus silvestris StLB046	6.32%	
	Pseudomonas sp.	6.36%	
	Arthrobacter sp.	5.60%	
	Fusobacterium necrophorum	0.01%	
	Uncultured *Porphyromonas* sp.	0.54%	
	Lysinibacillus sp.	1.74%	
	Bacillus oceanisediminis	1.34%	
	Carnobacterium maltaromaticum	1.21%	
	Exiguobacterium sp. AT1b	1.08%	
	Streptococcus sp.	0.92%	
	Parvimonas.uncultured bacterium	0.14%	
	Streptococcus salivarius subsp. thermophilus	0.72%	
	Psychrobacter sp.	0.66%	
	Pseudomonas vranovensis	0.55%	
	Helcococcus ovis	0.07%	
	Brochothrix thermosphacta	0.40%	
	Oceanobacillus profundus	0.37%	
	Escherichia-Shigella sp.	0.22%	
	Moraxella osloensis	0.27%	
	Ruminococcaceae.uncultured sp.	0.42%	
	Leuconostoc sp.	0.32%	
	Lactobacillus helveticus	0.33%	
	Leuconostoc lactis	0.30%	
	Peptoniphilus indolicus	0.02%	
	Ureaplasma diversum	0.20%	
	Acinetobacter sp.	0.30%	
SCE	*Escherichia coli*	(29.03%)	(Madhumeet & Pravesh, 2018)
	Staphylococcus spp.	(25.80%)	
	Bacillus spp.	(25.80%)	
	Streptococcus spp.	(9.67%)	
	Pseudomonas aeruginosa	(3.22%)	
	Micrococcus spp.	(3.22%)	
	Salmonella spp.	(3.22%)	

From the findings above, another two tables are formulated based on the average distribution of the most commonly isolated bacteria across the different clinical conditions which are Table 4 and Table 5. As for clinical endometritis cases, *T. pyogenes* was highly reported while subclinical endometritis showed high amount of *E. coli* in Table 4. Other than that, *T. pyogenes* and *E. coli* isolates were both highly found in metritis cases as compared to clinical and subclinical endometritis cases.

Table 4: Average distribution of the most commonly isolated bacteria across the different clinical conditions based on the number of cows detected positive.

Clinical condition	**Type of bacteria**					
	T. pyogenes	*E. coli*	*Staphylococcus* spp.	*Streptococcus* spp.	*Fusobacterium* spp.	*Bacteroides* spp.
M	57.35%	41.04%	31.67%	36%	32%	58.82%
CE	48.48%	24.83%	22.92%	34%	11.76%	20%
SCE	25%	32%	26%	7.5%	0%	0%

In Table 5, *Staphylococcus* spp. was reported to be higher than the other types of bacteria in cases of clinical endometritis. However, the difference was not as obvious as in Table 4. *E. coli* was still reported to be the highest in subclinical endometritis cases. Similarly, as in Table 4, *T. pyogenes* and *E. coli* were both highly discovered in metritis cases in comparison to other clinical conditions.

Table 5: Average distribution of the most commonly isolated bacteria across the different clinical conditions based on the percentage of isolates.

Clinical condition	**Type of bacteria**					
	T. pyogenes	*E. coli*	*Staphylococcus* spp.	*Streptococcus* spp.	*Fusobacterium* spp.	*Bacteroides* spp.
M	21%	86%	0%	33%	28.03%	24.86%
CE	14.09%	16.78%	21.46%	10.91%	9.14%	4.3%
SCE	0%	29.03%	25.8%	1.64%	0.01%	0%

Four isolates of *Helcococcus ovis* and two isolates of *Fusobacterium necrophorum* were found in metritis cases (Cunha et al., 2019; Francis et al., 2019). A total of 84 *E. coli* isolates were discovered in 25 cows with clinical endometritis (Yang et al., 2016). Fusobacterium spp. shows the highest mean proportion (29.4%) among the other species reported in 12 metritic cows (Bicalho et al., 2017). In three different studies, Bacteroidetes phylum was found to be abundant in the case of metritis (51.1%, 53.1% and 60.3± 10.3% respectively) (Jeon, Cunha, et al., 2016; Jeon et al., 2015; Sicsic et al., 2018). Evidences of the present review showed that isolation rate of *Trueperella pyogenes* was higher associated with clinical endometritis than subclinical endometritis (28.4% vs. 9.5%) while *Staphylococcus* spp. was much more abundant in subclinical endometritis than the clinical form (22.6% vs. 14.6%) (Prunner et al., 2014). There was a study done using two types of samples; uterine flush samples and endometrial biopsies. In metritis case, *Fusobacteriaceae* was discovered to be high in both flush and biopsy samples (22% and 26% respectively) while in week 7 endometritis, *Mycoplasmataceae* was found to be the highest only in flush sample which was at 52% (Knudsen et al., 2015).

3.4 Geographical Distribution

The geographical data of each published papers were evaluated by country and continent as shown in Figure 4 and Figure 5 respectively. Most of the studies were published from United States with a total of nine over 36 papers and followed by China with a total of five papers. When the studies were evaluated by continent as seen in

Figure 5, most of the studies were published from North America with a total of 10 over 36 papers.

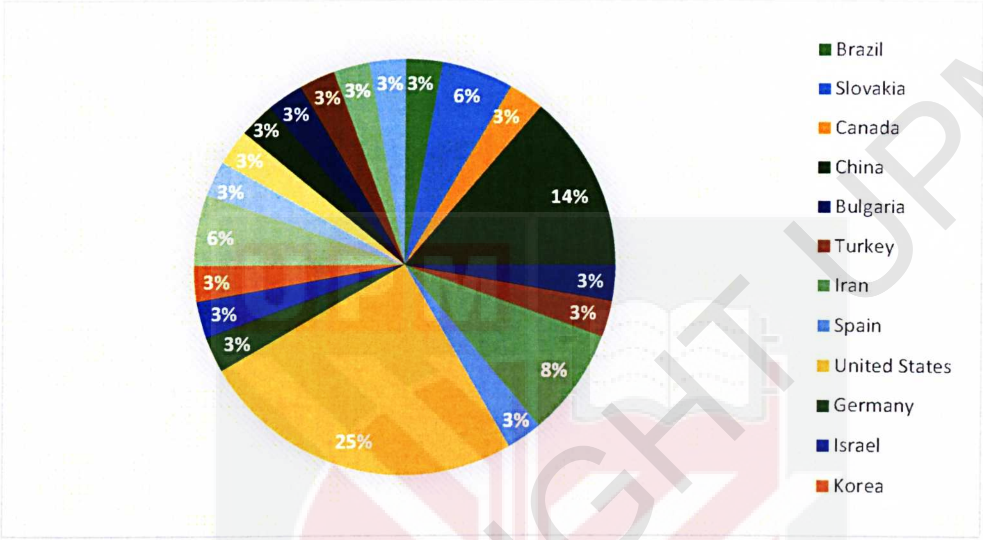

Figure 4: Geographical distribution of studies as evaluated by country.

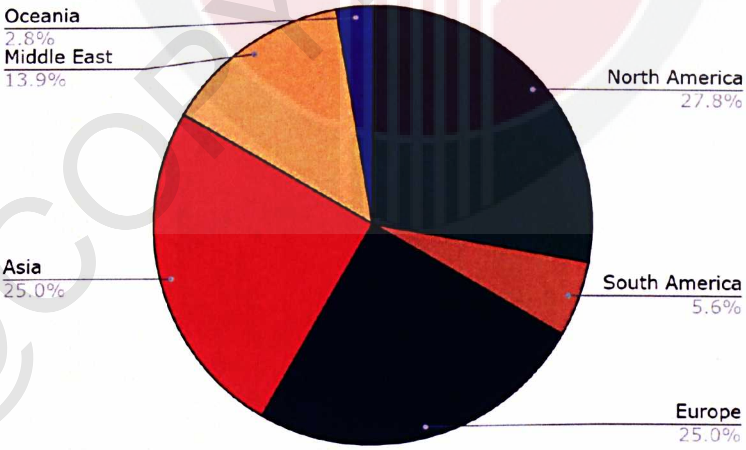

Figure 5: Geographical distribution of studies as evaluated by continent.

Geographical distribution of the major isolates as evaluated by continent are shown in Figure 6 – 11. Figure 6 shows that *T. Pyogenes* was highly reported in North America, Asia and Europe compared to other continents. However, it is noticeable that *T. Pyogenes* was discovered in all of the continents. Meanwhile, in Figure 7, *E. coli* was found to be highly reported in Asia.

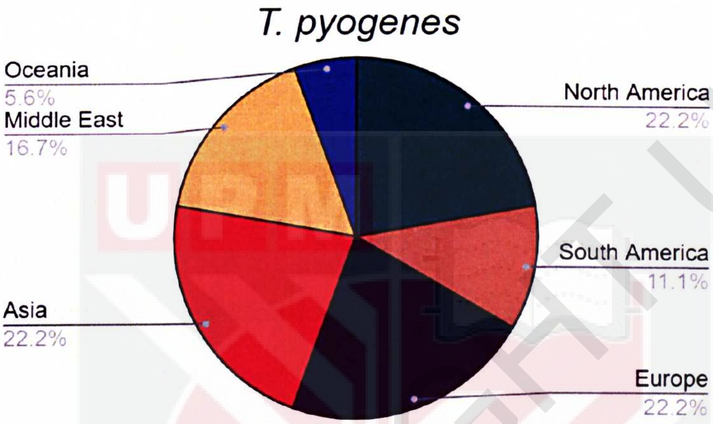

Figure 6: Geographical distribution of *T. pyogenes*.

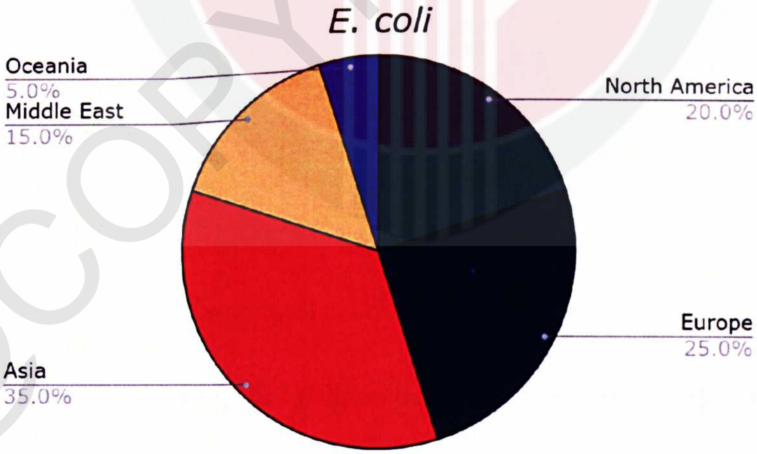

Figure 7: Geographical distribution of *E. coli*.

Similarly, *Staphylococcus* spp. and *Streptococcus* spp. were also highly discovered in Asia as shown in Figure 8 and Figure 9.

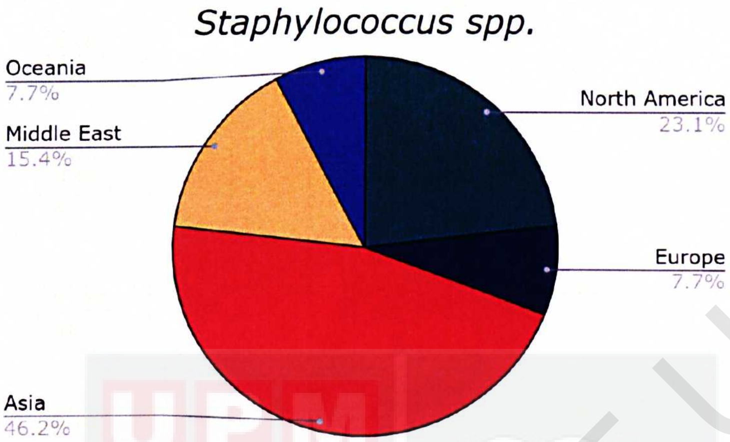

Figure 8: Geographical distribution of S*taphylococcus* spp.

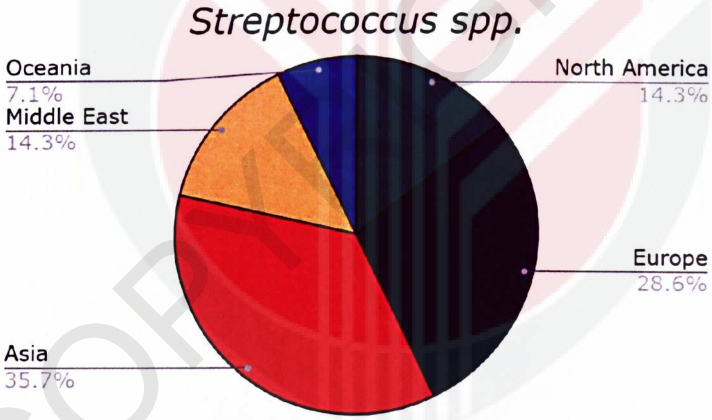

Figure 9: Geographical distribution of *Streptococcus* spp.

In contrast, Figure 10 and Figure 11 shows that *Fusobacterium* spp. and *Bacteroides* spp. were found to be the top in North America instead of Asia.

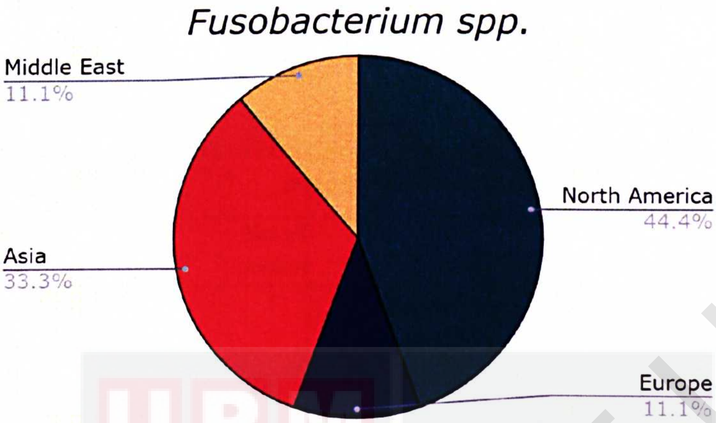

Figure 10: Geographical distribution of *Fusobacterium* spp.

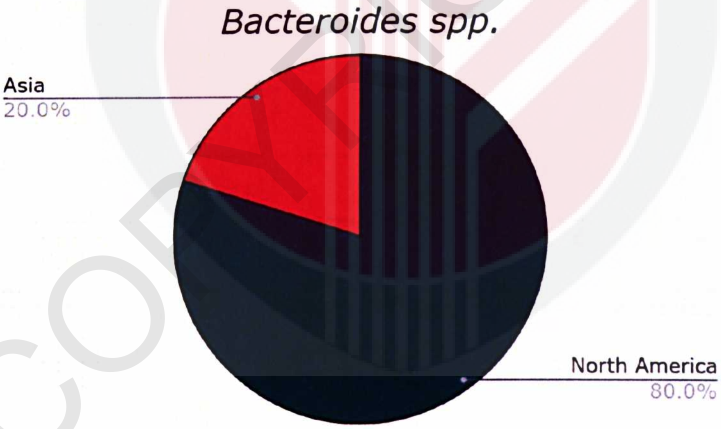

Figure 11: Geographical distribution of *Bacteroides* spp.

3.5 Risk Factors

Overall, 15 risk factors were reported to be associated with metritis, clinical and subclinical endometritis by some of the published papers. The risk factors are listed in Table 6 with the most common reported factors are retained placenta or fetal membrane, calving assistance such as in cases of twins, stillbirth or dystocia, and poor environmental conditions or sanitation.

Table 6: Risk factors reported to be associated with metritis, clinical and subclinical endometritis.

Risk factor	**No. of studies**	**Studies**
Retained placenta/fetal membrane	7	Brick et al., (2012), Mari et al., (2012), Takamtha et al., (2013), Ledgard et al., (2015), Jeon et al., (2015), Rezanejad et al., (2019), Schlegl et al., (2020)
Twins/stillbirth/dystocia (calving assistance)	6	Brick et al., (2012), Mari et al., (2012), Prunner et al., (2014) Ledgard et al., (2015), Rezanejad et al., (2019), Schlegl et al., (2020)
Poor environmental conditions/sanitation	5	Liu et al., (2013), Prunner et al., (2014), Knudsen et al., (2015), Kasimanickam et al., (2016), Schlegl et al., (2020)
Metabolic imbalance/disorder/disease	4	Brick et al., (2012), Mari et al., (2012), Ledgard et al., (2015), Wang et al., (2018)
Parity	2	Brick et al., (2012), Schlegl et al., (2020)
Post calving/parturition	2	Aghamiri et al., (2014), Ordell et al., (2016)
Host response	2	Jeon et al., (2016), Sicsic et al., (2018)
Calving season	2	Kasimanickam et al., (2016), Schlegl et al., (2020)
Cow perineal hygiene score	2	Brick et al., (2012), Kasimanickam et al., (2016)
Presence of *T. pyogenes* (CE)	2	Prunner et al., (2014), Wang et al., (2018)
BCS < 3.0	1	Paiano et al., (2021)
Non-medicinal bath measure	1	Liu et al., (2013)
Unusual green thick fodder supplies	1	Liu et al., (2013)
Intensive or pasture system	1	Kasimanickam et al., (2016)
Subclinical hypocalcemia (APM)	1	Credille et al., (2014)

3.6 Antimicrobial Sensitivity Test (AST) Findings

Out of 36 papers included in this review, only eight papers reported their AST findings on the microbes that were associated with metritis, clinical and subclinical endometritis as shown in Table 7. Figure 12 shows the comparison made between two out of the three studies on *T. pyogenes* antimicrobial resistance on the usage of three similar types of antibiotics which are amoxicillin, ampicillin and penicillin.

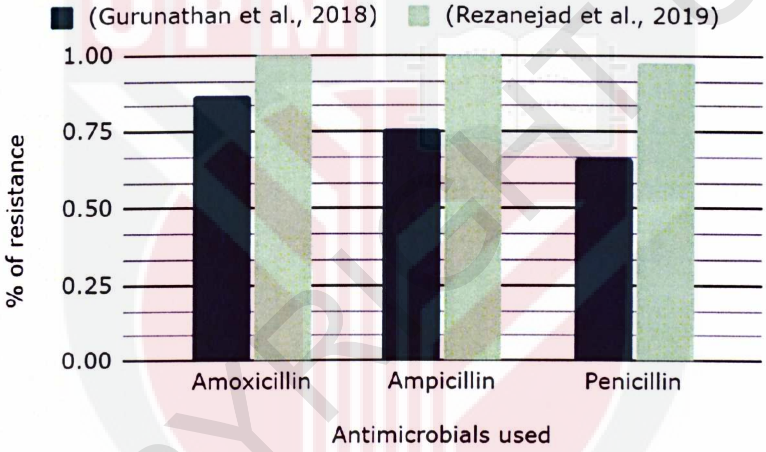

Figure 12: Percentage of *T. pyogenes* resistance against different antimicrobials by two different studies.

Table 7: AST findings identified from some of the published papers.

Bacterial isolates	Resistance	Study
Trueperella pyogenes	Ampicillin (100%), amoxicillin (100%), gentamicin (97.56%), penicillin (97.56%) and cefalexin (97.56%)	(Rezanejad et al., 2019)
Trueperella pyogenes	Trimethoprim sulfamethoxazole (72.3%), azithromycin (38.5%), erythromycin (36.9%), streptomycin (33.8%), tylosin (27.7%), enrofloxacin (17%), tetracycline (10.8%) and ciprofloxacin (9.2%)	(Ashrafi Tamai et al., 2018)
Prevotella melaninogenica and *Trueperella pyogenes*	*P. melaninogenica* were resistant to ampicillin (90.0%), cefalotin (79.0%), sulfamethoxazole/trimethoprim (65.2%), ciprofloxacin (54.6%), oxolinic acid (45.4%), gentamicin (43.8%), chloramphenicol (40.0%), cefotaxime (23.8%), ceftazidime (18.8%), amoxicillin/clavulanic acid (10.0%), and aztreonam (5.0%). *T. pyogenes* isolates exhibited particularly high levels of resistance to chloramphenicol (100%), amoxicillin (86.9%), ampicillin (76.1%), florfenicol (69.7%), penicillin (66.1%), oxytetracycline (64.2%), and tetracycline (50%).	(Gurunathan et al., 2018)
Escherichia coli, Staphylococcus species, Bacillus species, Streptococcus species, Pseudomonas aeruginosa, Micrococcus species, Salmonella species	Metronidazole (96.55%), Penicillin (80.52%), Cloxacillin (73.77%), Ampicillin (50.90%), Ceftriaxone (50.90%)	(Madhumeet & Pravesh, 2018)

Table 7: AST findings identified from some of the published papers (continued).

Bacterial isolates	**Resistance**	**Study**
Escherichia coli, *Staphylococcus* spp. and *Klebsiella* spp.	Ampicillin and Amoxycillin antibiotic (90.0%), Co-trimoxazole and Enrofloxacin (70.0%), Tetraacycline (50.0%) and Ceftriaxone and Gentamicin (40.0%)	(Yadav & Jadhav, 2018)
Escherichia coli	Ampicillin, carbenicillin, mezlocillin, amoxicillin and tetracycline (47.6–71.4%), followed by cefazolin, cefalotin, cefuroxime, cefotaxime, gentamycin, ciprofloxacin, ofloxacin, sulfamethoxazole and chloramphenicol (11.9–35.7%).	(Yang et al., 2016)
Staphylococcal isolates (*S. aureus* and CNS)	Penicillin (79.5%), ampicillin (71.7%) clindamycin (45.7%), tetracycline (52%) and erythromycin (56.7%).	(Zhao et al., 2014)
Corynebacterium spp., *Trueperella pyogenes*, *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Escherichia coli*, *Pasteurella* spp., *Actinobacillus* spp., *Enterobacter* spp., *Achromobacter* spp., *Klebsiella* spp., *Proteus* spp., *Citrobacter* spp., *Erysipelothrix* spp.	Gram-positive aerobic bacteria (*Corynebacterium* spp. until *Micrococcus* spp.) = oxytetracycline, streptomycin and sulfamethoxazole-trimethoprim were the 3 most resistant (43%, 25% and 27%, respectively). Gram-negative aerobic bacteria (*E. coli* until *Erysipelothrix* spp.) = oxytetracyclin, amoxicillin, ampicillin and streptomycin were the 4 most resistant (41%, 26%, 24% and 24%, respectively)	(Takamtha et al., 2013)

4.0 DISCUSSION

Sampling Methods for Bacteriology

There are a few sampling methods that were used to collect samples for bacteriology. Swab method was highly used from the published papers as it is the most accurate technique to acquire samples for specific and non-specific bacteria identification which can cause infection (Bonnett et al.,1993). The next preferred method of sampling was cytobrush as it can be performed rapidly and not complicated (Barlund et al., 2008; Kasimanickam et al., 2005). It was also claimed to be safe and effective to use, making it one of the best means of obtaining endometrial cytological samples (Oral et al., 2009).

Different sampling methods were employed across different clinical conditions as observed in Figure 3 with the swab method being the most frequent method used for metritis and clinical endometritis cases compared to subclinical endometritis and mixed condition. The presence of vaginal discharges in the cases of metritis and clinical endometritis makes it easier to obtain bacteriology samples by using swab method. However, it would be difficult to diagnose subclinical endometritis which should be done by measuring the proportion of neutrophils present in the samples collected. Hence, it is more suitable to do uterine flushing, or to use cytobrush in cases of subclinical endometritis.

Microbiota Distribution

A few types of bacteria were observed to be highly reported in metritis cases. These include *Bacteroides* spp., *T. pyogenes*, and followed by *E. coli*. Meanwhile, metritis cases in Table 5 recorded to be highly associated with *E. coli* at 86%. According to

Sheldon et al. (2006), pathogenic *E. coli* strains could cause endometrial surface diseases, namely postpartum metritis or endometritis due to the strains being more invasive and adherent in the endometrium.

From the findings, *E. coli* and *T. pyogenes* has always been reported to be high which actually shows that *E. coli* and *T. pyogenes* are abundant in the environment. Other than that, Knudsen et al. (2015) mentioned that *E. coli* and *T. pyogenes* are considered to be crucial in the pathogenesis of metritis and endometritis, as what have been reported by Bicalho et al. (2012) where the cows with uterine diseases were found to have the virulence factors from *E. coli* and *T. pyogenes.* The results tabulated were also consistent with the previous study by Sheldon et al. (2006) where it was reported that over than 80% of postpartum cows are susceptible to uterine contamination by *E. coli*, *Fusobacterium* spp., *T. pyogenes*, *Streptococcus* spp., *Staphylococcus* spp., *Clostridium* spp., *Pasteurella multocida*, and *Bacteroides* spp.

Geographical Distribution

Studies were retrieved from 16 different countries, with majority of the studies were done in United States and followed by China. When the geographical distribution of the papers was grouped by continent, most of the published studies were reported to be from North America, Asia and Europe as shown in Figure 5. However, this might be due to the limitation of this study where more papers should be included in this review to avoid information bias.

It is important to note that some differences in these findings might be due to the different number of reports received from each continent. Less reported cases may not necessarily mean less prevalence of the bacteria in the respective region. These findings might also be different due to the isolates of interest of the conducted study or due to the environmental differences.

Risk Factors

A number of risk factors are associated with metritis, clinical and subclinical endometritis, with some of them were frequently reported by the published papers included in this study. As mentioned by previous studies, retained placenta can lead to postpartum uterine diseases due to increase bacterial contamination and uterine damage. It also provides favorable environment for bacterial growth, which makes it the most important risk factors associated with uterine infection (Potter et al., 2010).

In cases of calving assistance, cows with dystocia are said to have higher chances of suffering from uterine infections and elevated culling rate in the future compared to those that do not require assistance during calving (Rogers et al., 2004). Calving assistance were reported to give effect on the rate of possible contamination of the uterus post calving (Madoz et al., 2013). Other than that, poor environmental conditions and sanitation can further cause uterine diseases due to the presence of higher bacterial load compared to a cleaner environment. This could lead to the establishment of pathogenic bacteria such as *T. pyogenes* which was one of the risk factors associated with uterine diseases, specifically clinical endometritis.

AST Findings

Three out of eight papers reported the AST findings specifically against *T. pyogenes*. *T. pyogenes* was reported to be completely resistant against ampicillin, amoxicillin and chloramphenicol. *T. pyogenes* was also found to be highly resistant against other antimicrobials such as gentamicin, penicillin and cefalexin.

Comparison was made in two out of the three studies on *T. pyogenes* antimicrobial resistance on the usage of three similar types of antibiotics which are amoxicillin, ampicillin and penicillin as shown in Figure 12. There is an increasing trend of resistance in all three of the antibiotics from 2018 to 2019, which indicates that *T. pyogenes* has developed more resistance towards some antibiotics. According to Rezanejad et al. (2019), the increase in antibiotic resistance was mainly due to excessive use of antimicrobial, unfinished treatment, incorrect medication choice and antibiotic resistance genes transfer among bacteria.

However, there are actually reports of discrepancy shown where the study by Gurunathan et al. (2018) reported that *T. pyogenes* was 50% resistant against tetracycline, whereas the study by Ashrafi Tamai et al. (2018) discovered that the resistance of *T. pyogenes* against tetracycline was only 10.8%. This might have happened due to the different strains and virulence factors of *T. pyogenes* that are present in their respective regions.

As this review was made, there were a few limitations identified which were related to the papers included in this study, where some of the studies conducted have specific isolates of interest, only some of the studies reported the risk factors associated with metritis and endometritis, and the published papers did not specify the virulence factors of the isolates.

5.0 CONCLUSION AND RECOMMENDATION

The main objective of this study was to systematically review and identify the uterine microbiota of dairy cows associated with metritis, clinical and subclinical endometritis from different geographical areas, along with their antimicrobial sensitivity spectrum and the most important risk factors. From the review, *T. pyogenes* was found to be the top isolate associated with clinical endometritis while *E. coli* was connected to most of the subclinical endometritis cases. Various types of bacteria which includes gram-positive and gram-negative bacteria were likely to cause metritis in cows. Hence, treatment should target the use of broad-spectrum antibiotics. In the aspect of geographical distribution, *T. pyogenes* can be found in almost all continent. *E. coli*, *Staphylococcus* spp. and *Streptococcus* spp. were highly reported in Asia, whereas *Fusobacterium* spp. and *Bacteroides* spp. were highly discovered in Europe. Other than that, the common risk factor frequently reported to be associated with uterine diseases were retained placenta or fetal membrane. From the AST findings, increase in the trend of antibiotic resistance was observed, with *T. pyogenes* was found to be highly resistant against chloramphenicol, ampicillin and amoxicillin.

In order to retrieve more papers and to perform a more thorough study, it is recommended for future studies to be conducted using more databases and to extend the publication period of the study reports to be included in the review.

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